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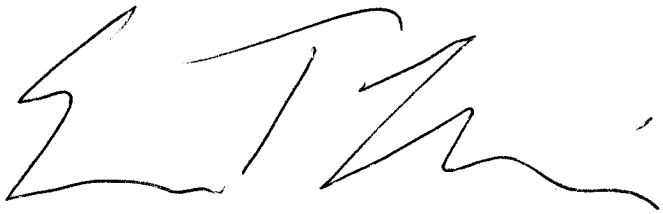

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ABSTRACT

Amygdala, Anxiety & α_1 Adrenoceptors: Investigations Utilizing a Rodent Model of Traumatic Stress

Sean T. Manion

Directed by He Li, M.D., Ph.D., Associate Professor Department of Psychiatry and Neuroscience Program, F. Edward Hebert School of Medicine, Center for the Study of Traumatic Stress, Uniformed Services University of the Health Sciences

Exposure to traumatic stress can result in post-traumatic stress disorder (PTSD) and other pathophysiological conditions. PTSD is characterized by a number of persistently heightened physiological and behavioral indicators, including exaggerated acoustic startle response (ASR) and alterations in processing of emotional memory. Similar effects can be seen in an animal model of traumatic stress in which stress results from restraint and inescapable tailshocks to rats. The basolateral amygdala (BLA) is an area known to be involved in the processing of emotional memory and startle modulation. Synaptic plasticity in the BLA is thought to play a key part in this memory formation, and therefore can be involved in subsequent stress related pathologies seen in PTSD.

The first part of this project used this model of traumatic stress to investigate the effects of prazosin, an α_1 adrenergic receptor (AR) antagonist, on stress induced elevation of ASR. Recent studies have shown the effectiveness of prazosin in treating PTSD. This investigation sought to determine its effectiveness in reducing the effects of traumatic stress when given prior to stress. Male Sprague-Dawley rats were injected with 0.5 mg/kg

of prazosin 30 minutes before inescapable tail shock on three consecutive days. ASR testing was performed 1, 4, 7 and 10 days post-stress and compared to baseline and control values. Results show a significant reduction of ASR hyperarousal in the pre-stress injection group. Pre-stress treatment with lower levels of prazosin (0.25, 0.1 and 0.05 mg/kg) showed similar reduction in ASR hyperarousal due to stress.

The second part of this project sought to investigate α_{1A} adrenoceptor involvement in long-term potentiation (LTP) in the BLA and to determine the effects of traumatic stress on this type of plasticity. In the BLA of control animals, the α_{1A} AR specific agonist A61603 (1 μM) completely abolished theta-burst stimulation-induced LTP. In animals previously exposed to a repeated restraint and tailshock stress protocol, only a partial reduction of LTP was detected in the presence of A61603. This blocking effect of A61603 on LTP in control animals was occluded in the presence of α_{1A} AR specific antagonist WB4101 (1 μM), while the antagonist did not eliminate the partial reduction of LTP in stressed animals. These findings suggest a possible mechanism contributing to the emotional memory in PTSD and support that the α_{1A} ARs can be a specific pharmacological target in PTSD.

Taken together, these findings offer both the possibility of preventative treatment for certain physiological symptoms of PTSD by administering prazosin prior to stress, as well as the potential involvement of α_{1A} ARs in the BLA in the emotional memory aspects of PTSD.

Amygdala, Anxiety & α_1 Adrenoceptors: Investigations Utilizing a Rodent Model of Traumatic Stress

by

Sean T. Manion

Dissertation submitted to the faculty of the Graduate Program in Neuroscience of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy 2006

DEDICATIONS

To the men and women of the United States Armed Forces who stand ready on my behalf. I am grateful.

To Mnemosyne, the kindest of muses when she visits, yet the cruelest of gods when she leaves. Though you will not remember.

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LIST OF ABBREVIATIONS

Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)

Acoustic startle response (ASR)

Adrenergic receptor (AR)

Artificial cerebrospinal fluid (ACSF)

Basolateral nucleus of the amygdala (BLA)

Central nervous system (CNS)

Central nucleus of the amygdala (Ce)

Circumventricular organs (CVO)

Corticosterone (Cort)

Dopamine (DA)

Epinephrine (EPI)

External capsule (EC)

Excitatory post-synaptic potential (EPSP)

Gamma-amino butyric acid (GABA)

Hertz (Hz)

Hypothalamic-pituitary-adrenocortical (HPA)

Lateral hypothalamus (LH)

Long-term depression (LTD)

Long-term potentiation (LTP)

Medial geniculate nucleus (MGN)

N-methyl-D-aspartate (NMDA)

Norepinephrine (NE)

Nucleus reticularis pontis caudalis (RPC)

Parabrachial nucleus (PB)

Paraventricular nucleus of the hypothalamus (PVN)

Periaquaductal grey (PAG)

Perirhinal cortex (PERI)

Phospholipase C (PLC)

Piriform cortex (PIR)

Post-traumatic stress disorder (PTSD)

Posterior intralaminar nucleus (PIL)

Protein kinase (PK)

Theta-burst stimulation (TBS)

Tyrosine hydroxylase (TH)

CHAPTER 1

Introduction

The mammalian nervous system has developed, through evolution, a quick response system to help deal with stress or threats from the external environment, as well as internal imbalances. This system, the sympathetic division of the autonomic nervous system, can quickly initiate changes in a number of different physiological systems in order to better enable the organism to deal with the stressor (by fight or flight, for example) and increase its likelihood of survival. Given appropriate levels of threat (life-threatening or repeated), long term changes can also occur that allow the animal to be hypervigilant for signs of the return of a previous threat. This adaptation better enables the animal to detect and possibly avoid potentially stressful or traumatic situations (Goldstein, 1995). Although beneficial in this regard, these long-term changes can become pathophysiological, becoming themselves a threat to the animal. In humans, this excess activity of the stress response system may result in a variety of disorders.

Post-traumatic Stress Disorder

Some of an organism's responses to stress, while useful in the short term, can become pathological in the long term. The stress associated with trauma and chronic stress is most often connected with these pathologies which can develop into a variety of anxiety disorders. One of these, post-traumatic stress disorder (PTSD; Table 1), is closely tied to these traumatic or chronically stressful conditions. It can arise from any number of

potentially life threatening events, from natural disasters to personal assaults, and it is most often associated with the stress of combat. Some individuals with certain predispositions, such as earlier exposures to trauma, can be more prone to develop PTSD following traumatic events.

PTSD may result from an event in which a person is seriously injured, witnesses death or serious injury to others, or perceives the threat of death or serious injury. Combat exposure, personal assault, terrorism, automobile accidents, and natural disasters are some of the most common causes. Reports indicate that from 5 to 30% of people exposed to such events will develop this disorder, and repeated exposure to these types of events increases this likelihood. In the U.S. approximately 3.6% of adults will suffer from PTSD in a given year (Kessler, 2002). Symptoms include flashbacks, nightmares, sleep disturbances, anxiety, irritability and anger, as well as increased blood pressure and heart rate and heightened arousal. These symptoms can occur independently, but stimuli associated with the traumatic event will often trigger onset of the symptoms. For this reason, avoidance of places and objects reminiscent of the event is quite common. Symptoms may first develop anytime from a few months to years after the event, and can last for months, years or even a lifetime, with or without treatment (Lopez-Ibor, 2002).

Table 1. DSM-IV Criteria for the Diagnosis of Posttraumatic Stress Disorder (309.81)

A. The person has been exposed to a traumatic event in which both of the following were present:
(1) the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others
(2) the person's response involved intense fear, helplessness, or horror. Note: In children, this may be expressed instead by disorganized or agitated behavior

B. The traumatic event is persistently reexperienced in one (or more) of the following ways:
(1) recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions. Note: In young children, repetitive play may occur in which themes or aspects of the trauma are expressed.
(2) recurrent distressing dreams of the event. Note: In children, there may be frightening dreams without recognizable content.
(3) acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated). Note: In young children, trauma-specific reenactment may occur.
(4) intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event
(5) physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three (or more) of the following:
(1) efforts to avoid thoughts, feelings, or conversations associated with the trauma
(2) efforts to avoid activities, places, or people that arouse recollections of the trauma
(3) inability to recall an important aspect of the trauma
(4) markedly diminished interest or participation in significant activities
(5) feeling of detachment or estrangement from others
(6) restricted range of affect (e.g., unable to have loving feelings)
(7) sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:
(1) difficulty falling or staying asleep
(2) irritability or outbursts of anger
(3) difficulty concentrating
(4) hyper-vigilance
(5) exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C, and D) is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Specify if:
Acute: if duration of symptoms is less than 3 months
Chronic: if duration of symptoms is 3 months or more

Specify if:
With Delayed Onset: if onset of symptoms is at least 6 months after the stressor

From the American Psychiatric Association; DSM-IV

The cost of PTSD is considerable. Approximately 5.2 million Americans suffer from it in any given year, costing more than an estimated 50 billion dollars. While some develop symptoms shortly after the traumatic event, others take years to develop them. Conversely, some may see their symptoms fade, regardless of treatment, over time; others will be afflicted for life. There are both cognitive/behavioral and pharmacological treatments available, but neither is fully effective in all or even most individuals. Complicating matters is an unwillingness to seek treatment for what has historically been sometimes thought of as merely a character flaw or personal weakness. These factors make the search for the underlying mechanisms and perhaps better treatment for PTSD a worthwhile endeavor.

PTSD and Exaggerated Acoustic Startle Response

There are a number of different symptoms of PTSD that are comparable to dysfunctions that can be induced by stress in rats (Table 2). In particular, the exaggerated startle response is one similarity that is widely used in stress studies and evaluations of PTSD. The startle response is a reflex that can be elicited by a variety of intense stimuli: tactile, acoustic or vestibular. In the case of the acoustic startle response (ASR), a startle is elicited with a sudden noise burst and measured as the peak of the startle amplitude.

Table 2. Comparison of Symptoms in PTSD to Stress-Induced Dysfunction in Rats

PTSD in Humans	Inescapable tail-shock model of stress in rats
Difficulty falling or staying asleep, nightmares [1;2]	Altered sleep patterns [3]
Psychomotor numbness [4;5]	Persistent behavioral abnormalities i.e. suppressed open-field activity [6;7]
Poor concentration; memory deficits [8-10]	Deficits in escape learning [11]
Hypervigilance and/or exaggerated startle response [12;13]	Exaggerated startle response [14]
Hyper-responsiveness of the noradrenergic system [15;16]	Hyper-responsiveness of the noradrenergic system [17]

[1] Maher et al., 2006, [2] Raskind et al., 2003, [3] Adrien et al., 1991, [4] Epstein et al., 1998, [5] Jatzko et al., 2006, [6] Minor et al., 1994, [7] Pare et al., 1994, [8] Bremner et al., 2004, [9] Milad et al., 2006, [10] Moradi et al., 1999, [11] Maier et al., 2001, [12] Famularo et al., 1990, [13] Mouren-Simeoni et al., 1993, [14] Servatius et al., 1995, [15] Orr et al., 2002, [16] Maes et al., 1999, [17] van der Wal et al., 1985

This metric, the ASR, can be used to measure the effect of a variety of different modifiers to the startle response, from drugs to environmental conditions. It has repeatedly been seen that stress can cause an exaggeration of ASR in both humans and animals. This effect of stress on ASR can then be used to evaluate the effectiveness of drugs given at different time points to further increase or to decrease the exaggerated ASR.

Amygdala Involvement in Stress Response and Emotional Memory

The amygdala is a key component of the brain's neuronal network that determines the emotional significance of external events and is involved in the processing of emotional memory (Davis, 1994; Buchel, 1998). Via efferent pathways to the hypothalamus, the amygdala also can trigger the neuroendocrine cascades that are part of the stress response (Habib et al., 2001; Pitkänen, 2000; Davis, 1992) and via reciprocal connections with the cerebral cortex and limbic structures it modulates the orchestration of the behavioral response to stress (Goldstein et al., 1996; Pitkänen et al. 2000). Therefore, understanding the changes in amygdala physiology and function induced by stress is critical to understand the pathophysiology of stress, and may aid the development of new therapeutic strategies for the prevention and treatment of stress-related affective disorders such as PTSD.

Synaptic plasticity closely associated with memory formation and can take several forms including long-term potentiation (LTP) and long-term depression (LTD). LTP can be produced after strong and/or repetitive activation of the amygdala during stressful emotional experiences. Similar long-term synaptic changes could be involved in the development and manifestation of PTSD in humans. Patients suffering from affective disorders display abnormal amygdala responsiveness to emotional stimuli (Rauch et al., 2000; George et al., 1993). It has also been demonstration that LTP in the amygdala can develop in parallel with fear conditioning, a model for emotional memory involved in disorders of fear and anxiety (Rogan et al., 1997). Investigation of the characteristics and mechanisms of synaptic plasticity in the amygdala and how these are affected by stress may help to better understand the biological mechanisms underlying PTSD and other associated disorders.

Norepinephrine and other neurotransmitter involvement

Norepinephrine (NE), also known as noradrenaline, is a catecholaminergic neurotransmitter that plays an important role in several different aspects of the stress response. Its synthesis, storage, release, reuptake, and receptor binding are each areas where short and long-term changes can occur, altering the stress response. By examining these areas where NE function can be affected, a better understanding of changes in the stress response can be obtained.

The adrenergic receptors (ARs) are a group of G-protein coupled receptors that actively bind the NE and epinephrine (EPI). There are currently three known families of ARs each with three subclasses: α1 (α_{1A}, α_{1B}, α_{1D}), α2 (α_{2A}, α_{2B}, α_{2C}), and β (β_1, β_2, β_3). G-proteins bound to the various subtypes can alter activity by way of adenylate cyclase, phosphoinositol, phosolipase C, and calcium channels (Cotecchia et al., 2000). The different AR subtypes are found in a variety of roles in a number of different brain regions, including the amygdala.

The α_1 family subtypes are found widely distributed throughout the CNS, including such areas as cortex, hypothalamus, thalamus, amygdala and midbrain (Ferry et al., 1999). Within the amygdala, the three α_1 subtypes are distributed differently among the major nuclei. In the central and basolateral nuclei, α_{1A} receptors are expressed in moderate to high levels, whereas α_{1B} and α_{1D} receptors are expressed at low levels. Conversely, in the lateral nucleus, α_{1B} and α_{1D} receptors are expressed at very high levels and α_{1A} receptors at much lower levels (Day et al., 1997).

During stress, there is a strong enhancement of NE release in the BLA (Galvez et al., 1996; Stanford, 1995; Quirarte et al., 1998; Tanaka et al., 2000). The α_1 ARs have also been shown to have a role in stress and memory in the amygdala (Ferry et al., 1999) though their role is not as thoroughly understood. It has additionally been established that ARs can become desensitized after prolonged exposure to agonists (Yang et al., 1999; Chalothorn et al., 2002).

NE can modulate GABAergic inhibition primarily via the α_1 subtype of adrenergic receptors (Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles et al., 1996; and Kawaguchi and Shindou, 1998). Chronic stress in rats reduces the expression of these receptors in the hypothalamus and brain stem (Miyahara et al., 1999), though its effect on levels in the amygdala is not yet known. Blockade of these receptors in rats increases depressive behavior (Stone and Quartermain, 1999). Furthermore, we have seen that repeated restraint and tailshock stress produces a severe impairment in the α_{1A} AR-mediated facilitation of GABA release in the BLA, indicating that stress impairs the function of α_{1A} ARs (Braga et al., 2004).

Different lines of evidence point to the possibility that the function of the GABAergic system may be impaired by stress. First, in a number of brain regions, benzodiazepine receptor binding is altered by stress (Lippa et al. 1978; Medina et al. 1983; Miller et al. 1987; Weizman et al. 1989; Bremner et al. 2000). Second, there are many psychotropic drugs that are effective in the treatment of emotional disorders that target or influence GABAergic transmission. Third, stress exacerbates the frequency of seizures in epileptic patients (Temkin and Davis, 1984; Frucht et al., 2000). Fourth, in certain stress-related psychiatric disorders, the amygdala exhibits higher than normal levels of basal activity (Abercrombie et al., 1998; Drevets, 1999), or exaggerated responses to fearful stimuli (Rauch et al., 2000; Villarreal and King, 2001). Since the GABAergic system is a primary regulator of neuronal excitability, pathophysiological changes in GABAergic transmission may underlie the amygdala's hyper-responsiveness and hyperexcitability in these emotional disorders.

Hypotheses & Rationale

Hypothesis 1: *Administration of α_1 adrenoceptor antagonists 30 min prior to a period of intense stress, to block these receptors from excessive NE activation, will prevent stress-induced alterations in acoustic startle response.*

Hypothesis 2: *An excessive release of NE at the time of a traumatic event is the mechanism responsible for the long-lasting impairment of the α_{1A} adrenoceptor-mediated facilitation of synaptic inhibition in the basolateral amygdala. This would result in hyperexcitability of the amygdala, causing facilitation of LTP induction in the BLA of stressed rats.*

PTSD is associated with repetitive, vivid memories of traumatic events such as combat, natural or man-made disasters, terrorist attacks and other traumatic events. The participation of the amygdala in PTSD and other emotional aspects of behavior is becoming increasingly well established in humans and animals (Cahill et al., 1995; Davis, 1994; Davis et al., 1997; Rogan and LeDoux, 1996). The amygdala has extensive connections with the cerebral cortex, the hypothalamus, the limbic system, and the brain stem; and is capable of integrating information that is necessary for the proper execution of the stress response. Little is known, however, of the nature of synaptic function in the amygdala after exposure to stress.

The paradigm of conditioned fear can be assessed by measurement of the acoustic startle response, a primitive reflex that is part of an animal's response to threat. The natural pattern of behaviors produced by conditioned fear can be blocked by lesions of specific nuclei within the amygdala (Gallagher et al., 1990) and evoked by electrical stimulation of the amygdala (Koch & Ebert, 1993; Rosen & Davis, 1988). In addition, short and long-term changes in neuronal plasticity in the amygdala have been considered an important process of conditioned fear (Davis et al., 1997; McKernan & Shinnick-Gallagher, 1997; Rogan et al., 1997).

An increase in both urinary and plasma levels of norepinephrine and epinephrine have been demonstrated following exposure of PTSD patients to traumatic reminders. An increase of startle responsiveness in these patients appears to be mediated by the increased release of catecholamines that act on target tissues via α and β ARs (Bremner et al., 1996; Southwick et al., 1999). In an animal model of PTSD which uses inescapable tail shock as a stressor, stressed rats exhibit a similar increase in turnover and release of NE in the cerebral cortex and in several subcortical areas, including the amygdala, the hippocampus, the hypothalamus and the locus coeruleus (Tsuda et al., 1989; Southwick et al., 1999; Bremner et al., 1996; Li et al., 1998). Increased release of NE in the amygdala is associated with behaviors which are typically seen during states of fear, suggesting that NE may play a role in mediating fear responses in amygdala circuits (Servatius et al., 1995).

It has been hypothesized that the hyperactivity and hyper-responsiveness of the amygdala associated with certain affective disorders, such as PTSD, is due to the loss of proper cortical modulation of the amygdala, and/or due to an intrinsic lower threshold of amygdala response to emotionally significant stimuli (Villarreal & King, 2001). Our previous studies suggest that a reduction in GABAergic transmission due to the loss of the α_{1A} AR-mediated facilitation of GABA release may be one of the mechanisms responsible for the apparently reduced threshold of amygdala's activation in these affective disorders (Braga et al., 2004). We postulated that an excessive release of NE at the time of a traumatic event is the mechanism responsible for the long-lasting impairment of the α_{1A} adrenoceptor-mediated facilitation of synaptic inhibition in the basolateral amygdala. This results in hyperexcitability of the amygdala, causing exaggerated responses to fearful stimuli and therefore exaggerated startle reflex and anxiety, which can also lead to weight loss. Excessive release of NE at the time of a traumatic event can also lead to elevated corticosterone levels as it has been previously demonstrated that NE acting via α_1 adrenoceptors in the amygdala activates the hypothalamo-pituitary-adrenocortical axis (Feldman and Weidenfeld, 1996). Administration of an α_1 adrenoceptor antagonist 30 minutes prior to a period of intense stress, to block/protect these receptors from excessive NE activation, will prevent stress-induced alterations in the amygdala's physiology and function.

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CHAPTER 2

Prazosin administered prior to inescapable tail shock blocks subsequent exaggeration of acoustic startle response in rats

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Exposure to traumatic stress can result in a number of pathophysiological conditions, including post-traumatic stress disorder (PTSD). PTSD is characterized by a number of persistently heightened physiological and behavioral indicators, including increased sensory arousal and exaggerated acoustic startle response (ASR). Similar effects can be seen in an animal model of PTSD in which stress results from restraint and inescapable tailshocks to rats. The present study used this animal model to investigate the effects of prazosin, an α_1 adrenoceptor antagonist, on stress induced elevation of ASR. To investigate this, male Sprague-Dawley rats were injected with 0.5 mg/kg of prazosin 30 minutes before inescapable tail shock on three consecutive days. ASR testing was performed 1, 4, 7 and 10 days post-stress and compared to baseline and control values. Results show a significant reduction of ASR hyperarousal in the group treated with prazosin prior to stress compared to vehicle treated stressed animals and controls. Pre-stress treatment with lower levels of prazosin (0.25, 0.1 and 0.05mg/kg) showed similar results. These findings further implicate an α_1 adrenoceptor role in the pathophysiological response to traumatic stress and suggest a potential preventative role for prazosin.

Keywords: Prazosin, α_1 adrenoceptor antagonist, Traumatic stress, Inescapable tail shock, Acoustic startle response, Sprague-Dawley rats, Body weight, Behavior

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Introduction

Stress triggers biological and behavioral responses that enable the organism to cope with the stressor. Extreme or chronic stress can cause a variety of detrimental effects that may lead to lasting pathophysiology associated with the systems involved in the stress response (Vermetten and Bremner, 2002; McEwen, 2002). In humans, this can result in affective disorders including acute stress disorder (ASD) and post-traumatic stress disorder (PTSD) (Osuch et al., 2004). Research has shown that many different neurotransmitter systems play significant roles in the stress response, including the noradrenergic system (Southwick et al., 1999). This system may therefore be a potential target for prevention or treatment of stress-induced pathophysiologies.

Within the noradrenergic system, norepinephrine (NE), also known as noradrenaline, is a catecholaminergic neurotransmitter that plays an important role in the stress response, both peripherally and centrally. The noradrenergic system in the brain is centered in the locus coeruleus (LC) which has efferents connecting it to a number of areas involved in the stress response. One particular brain structure is the amygdala, which has been implicated in mediating the fear response and regulating the emotional aspect of memory (Ledoux, 2000; Davis, 1998). Neurons projecting from the LC target a variety of adrenoceptor (AR) subtypes in the amygdala, generally classified as both alpha (α_1 and α_2) and beta (β_1, β_2) receptors. The subtypes of the α_1 family are found widely distributed throughout the CNS, including such areas as cortex, hypothalamus, thalamus, amygdala and midbrain (Miyahara et al., 1999). The amygdala in particular is a focus of

investigation with regard to its role in both the normal and the pathophysiological response to stress (McEwen, 2002).

During stress, there is a dramatic increase in NE activity in the amygdala resulting in the peripheral release of epinephrine from the adrenal glands (Stanford, 1995; Bremner et al., 1996; Galvez et al., 1996; Quirarte et al., 1998; Tanaka et al., 2000). Neuronal excitability in central adrenergic pathways is mediated via concurrent activation of α and β adrenoceptors to regulate behaviors in response to stressful stimuli. In the basolateral amygdala, α_{1A} adrenoceptor activation suppresses neuronal excitability by attenuating excitatory synaptic transmission and facilitating inhibitory synaptic transmission (Braga et al., 2004). In contrast, β adrenoceptor activation enhances neuronal excitability and memory storage by facilitating excitatory synaptic transmission (Huang et al., 1994; Ferry and McGaugh, 2000). The involvement of β ARs in the amygdala's role in the stress response has been widely established (McGaugh, 2002). It has been shown that there is an interaction between α_1 and β AR modulation in the amygdala (Ferry et al., 1999). Recently it was seen that blockade of α_1 ARs in the central amygdala could attenuate stress-induced reduction of social behavior independently of β involvement (Morilak et al., 2005). These findings point out the need for further investigation of the role of α_1 ARs in the response to stress.

An inescapable tail shock model of traumatic stress in rats has been shown to cause physiological and behavioral changes similar to those seen in humans with PTSD (Servatius, et al., 1995). Specifically, after exposure to a three day restraint and tailshock

protocol, rats show persistent hyperarousal (Servatius et al., 2000) and exaggerated ASR (Beck et al., 2002). ASR is the unconditioned response to brief, loud acoustic stimuli that can be seen across various mammalian species. It is frequently used to characterize the CNS response to various pharmaceuticals. The cross-species validity of these measurements allows them to be used to evaluate animal models of various pathophysiologies and to explore the efficacy of pharmaceuticals to humans (Faraday & Grunberg, 2000). Exaggerated ASR is a common symptom of PTSD and is thought to be related to the increased NE release associated with the stress (Southwick et al., 1997).

Widely used for the treatment of hypertension for more than 50 years, prazosin is the prototypic quinazoline-bearing α_1 selective AR antagonist (Antonello et al., 2005). It has also been shown to act in the CNS upon peripheral delivery (Rogawski and Aghajanian, 1980, Menkes et al., 1981) based on its ability to readily cross the blood brain barrier. In rats, prazosin has been shown to block short and long term habituation effects of ASR (Leaton & Cassella, 1984), as well as to block apomorphine enhancement of startle (Davis et al., 1985). In humans, it has been shown to reduce trauma-related nightmares and sleep disturbances associated with PTSD (Raskind et al., 2000, Raskind et al., 2003) at dosages comparable to those used to treat hypertension. Prazosin's effectiveness, combined with its established safety and minimal side effects, make it an ideal candidate for further study to determine its ability to intervene in the pathophysiologies that can result from exposure to traumatic stress.

This study was undertaken to test the hypotheses of whether administration of prazosin before stress would affect stress-induced exaggerations of ASR in rats. Results showed for the first time that prazosin was effective at blocking these stress-induced exaggerations of ASR when administered before stress. These results give further insight into the mechanisms underlying lasting changes in response to stress, and may indicate that prazosin could serve as a potential prophylactic agent to block the development of ASD and PTSD in situations where extreme or traumatic stress can be foreseen, such as combat or disaster relief.

Methods

Subjects and Experimental Design

Subjects were 120 male Sprague-Dawley rats weighing 125-175g at the beginning of the experiments (Taconic Farms, Germantown, NY, USA). Animals were pair-housed in standard polypropylene cages on hardwood chip bedding in a climate controlled environment (23°C, 70% humidity) with a reverse light/dark cycle (lights on at 1730h) ad libitum access to food and water. Three different experiments were conducted. In Experiment 1, 42 rats were divided into two groups, stressed (n=15) and control (n=27). In Experiment 2, 48 rats were divided into four groups, stressed/saline (n=12), stressed/prazosin 0.5 mg/kg (n=12), control/saline (n=12) and control/prazosin 0.5 mg/kg (n=12). In the Experiment 3, 30 rats were divided into five groups, control/saline (n=6), stressed/saline (n=6), stressed/prazosin 0.25 mg/kg (n=6), stressed/prazosin 0.10 mg/kg

(n=6) and stressed/prazosin 0.05 mg/kg (n=6). All animal experiments were performed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) directives after obtaining the approval of the USUHS Institutional Animal Care and Use Committee.

Acclimation

In order to avoid undue stress to the animals and better focus on the effects of intentional stress on specific groups, all animals were handled for approximately 5 min/day for two days prior to acclimation. Animals were weighed throughout the experiment, both as a physiological measure and as a metric for balancing the groups. Animals had to be a minimum of 150 g at the time of baseline procedure in order to be included. Animals were acclimated to the acoustic startle equipment (details below) for three consecutive days, one day without sound followed by two days with sound. This acclimation was finished three days prior to baseline recordings in order to avoid desensitization effects.

Baseline

A baseline recording of acoustic startle response (details below) was taken for each of the animals on the day prior to beginning the stress procedure.

Stress

Stress exposure consisted of a two-hour per day session of immobilization and tail-shocks for three consecutive days. Stressing was done during the dark or active phase of the light-dark cycle. Animals were restrained by being wrapped in a cloth jacket and having their head and torso immobilized in a ventilated plexiglass tube. Forty electric shocks (2-3mA, 3s duration; Animal Test Cage Grid Floor Shocker, Coulbourn Instruments, USA) were delivered to their tails at semi-random intervals of 150 to 210s (Graphic State Notation software, Habitest Universal Link, Coulbourn Instruments, USA). This stress protocol was adapted from the learned helplessness paradigm in which animals undergo an aversive experience under conditions in which they cannot perform any adaptive response (Seligman and Maier 1967; Seligman and Beagley 1975). The duration of stress was based on previous demonstrations that repeated stress sessions for three days is more effective than a single stress session in producing lasting physiological and behavioral abnormalities, such as elevations in basal plasma corticosterone levels, exaggerated acoustic startle responses and reduced body weight (Servatius et al. 1995; Ottenweller et al. 1989). Previous studies have shown that additional stress sessions, beyond the three days, do not appear to produce greater physiological and/or behavioral changes (Servatius et al. 1995; Ottenweller et al. 1989).

Drugs

Prazosin hydrochloride (Sigma Chemical Co., St. Louis, MO), an α_1 specific adrenergic antagonist, in a 0.9% sodium chloride solution vehicle (Abbott Laboratories, North Chicago, IL) or vehicle alone was delivered by intraperitoneal injection (1mL latex free syringe with 27G ½-inch needle, B-D, Franklin Lakes, NJ). Prazosin was delivered to the drug group animals, both stress and control, at a dosage of 0.5mg/kg, 30 minutes prior to the stress procedure on each of the three stress days in the initial prazosin studies. Saline vehicle was delivered to the appropriate groups at the same time. In the dosage experiment, dosages of 0.05, 0.1 or 0.25 mg/kg were delivered to associated groups 30 minutes prior to each stress session, with vehicle being delivered to appropriate groups at the same time.

Acoustic Startle

ASR testing was conducted with a Startle Response Acoustic Test System (Coulbourn Instruments, PA). This system consists of four weight-sensitive platforms in a sound-attenuated chamber, though only one platform was used at a time. This was done to reduce interactive effects between animals. The animals' movements in response to stimuli were measured as a voltage change by a strain gauge inside each platform and were converted to grams of body weight change following analog to digital conversion. These changes were recorded by an interfaced computer as the maximum response occurring within 200 ms of the onset of the startle-eliciting stimulus. All acoustic stimuli

were administered by an amplified speaker mounted 24 cm above the test cage. During testing, animals were individually placed in holding cages (14.5 × 7 × 6.5 cm) that were small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small movements. These were then placed on the weight sensitive platform.

Following placement of the animal into the chamber, the chamber lid was closed, leaving the subject in darkness. A 3 minute adaptation period occurred in which no startle stimuli were presented. Startle stimuli consisted of 110 dB sound pressure level (unweighted scale; re: 0.0002 dynes/cm^2) noise bursts of 20 msec duration sometimes preceded 100 ms by 68 dB 1 kHz pure tones (pre-pulses). Decibel levels were verified by a Radio Shack Sound Meter (El Paso, TX). Each stimulus had a 2 ms rise and decay time such that onset and offset were abrupt, a primary criterion for startle. There were six types of stimulus trials: 110 dB alone, with pre-pulse, pre-pulse alone and no stimulus. Each trial type was presented eight times. Trial types were presented in random order to avoid order effects and habituation. Inter-trial intervals range randomly from 15 to 25 s. All animals were tested 1, 4, 7 and 10 days following the final day of the stress procedure.

Data Analysis

Each animal's responses were averaged within trial type. Trials during which no stimuli were presented were used to control for normal subject movements on the platform. Amplitudes to each trial type were derived by subtracting grams of platform

displacement on the no-stimulus trials (i.e., the body weight of each subject) from platform displacement in response to specific stimuli. The remainder from this calculation represented the amount of platform displacement related to the stimulus (e.g., 110 dB, 110 dB with pre-pulse). Analyses of variance (ANOVA) for repeated measures with factors of stress status and drug dosage and test day as the repeated measure were performed. These global tests were used to reveal interactive effects among the variables. These results were followed with ANOVAs on individual test days to determine the effect of stress and drug treatments. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Stress and Weight

As seen in Figure 1, the three day stress protocol of restraint and inescapable tail-shock showed an effect of stress status on body weight. As was consistent with previous experiments, Student's t-tests showed that change in percentage of baseline body weight was significantly higher in the control animals (n=27) as compared to the stressed animals (n=15) on day one [$t(40)=6.32$; $p<0.001$], day four [$t(40)=5.34$; $p<0.001$], day seven [$t(40)=4.05$; $p<0.001$] and day ten [$t(40)=2.71$; $p=0.010$] following stress. Similar weight differences existed for all stress versus control groups, with no significant effect of drug on weight (data not shown).

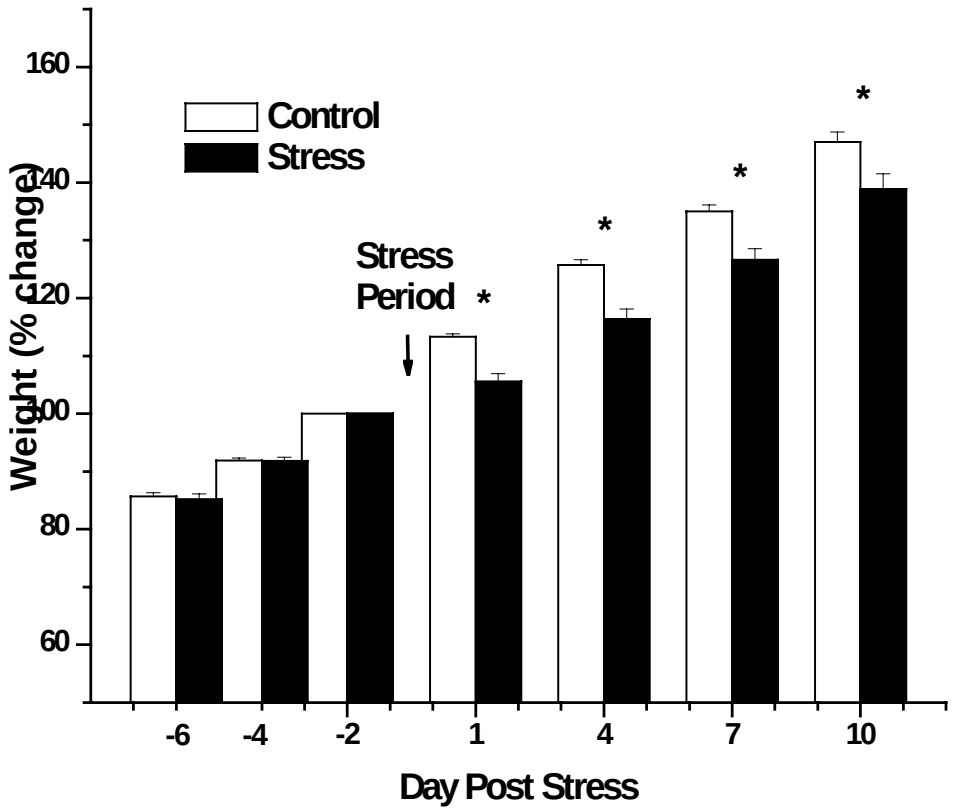

Figure 1 – Percentage of body weight immediately prior to stress procedure (group means ± SEM) for control and stressed rats on 4, 2 and 0 days preceding and 1, 4, 7 and 10 days following the three day stress procedure. Asterisks (*) indicate significant differences between the two groups (n=27 control, 15 stress; $p<0.05$).

Experiment 1; Stress and ASR

Acoustic startle testing was used to test hyperarousal following stress (Fig.2). Repeated measures ANOVA showed that there was an significant effect of day [$F(4,156)=5.70$, $p<0.001$] and a significant interaction of day x stress [$F(4,156)=2.86$, $p=0.025$]. Student's t-test for specific days showed that ASR was significantly higher in the stressed animals (n=15) as compared to the control animals (n=26) at day seven [$t(39)=2.11$; $p=0.041$] and day ten [$t(39)=2.21$; $p=0.033$] following stress.

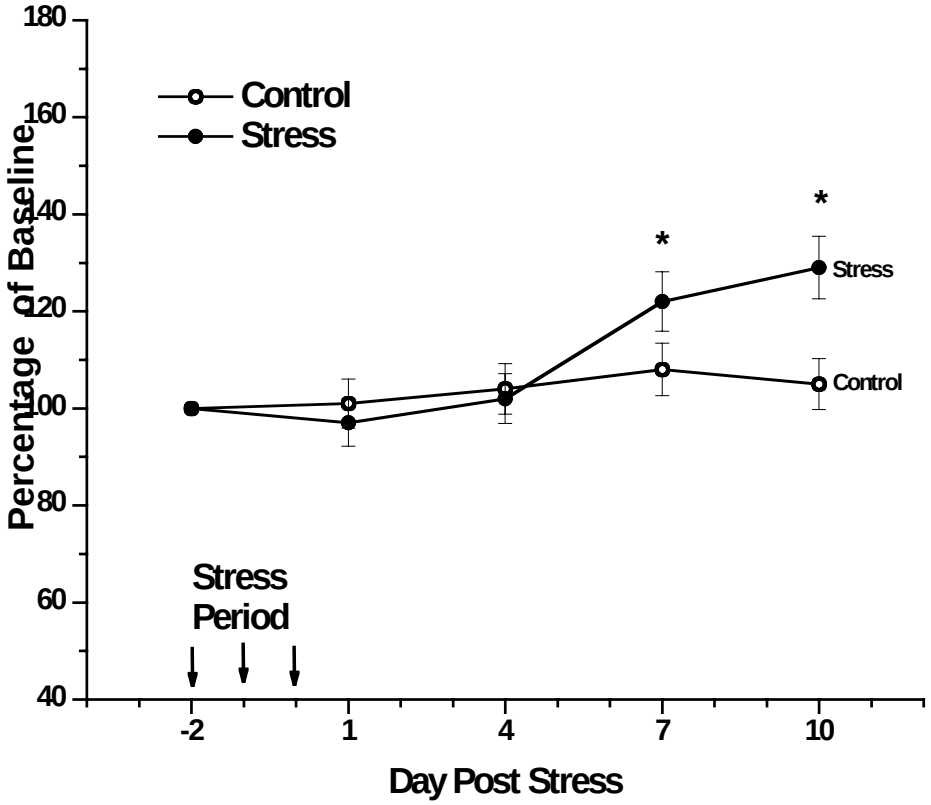

Figure 2 – Percentage of pre-stress baseline of acoustic startle (group means ± SEM) for groups of stressed and control animals. Asterisks (*) indicate significant differences in ASR between stressed animals and control (non-stressed) animals (n=26 control, 15 stress; $p<0.05$).

Experiment 2; Pre-Stress Prazosin

In animals treated with 0.5mg/kg of prazosin 30 minutes prior to stress (Figure 3), ANOVAs for repeated measures with factors of stress status and drug dosage, and test day as the repeated measure showed an effect of day [$F(3.4,196)=2.3$, $p=0.07$], and significant interactions between day x stress [$F(3.4,196)=4.8$, $p=0.001$], day x drug [$F(3.4,196)=4.0$, $p=0.004$] and day x stress x drug [$F(3.4,196)=5.24$, $p<0.001$]. Tukey's post-hoc test on each day indicated that the stress/saline group differed from all other groups on days 4, 7 and 10 following stress ($n= 12$-15 for each group, $p<0.05$).

Acoustic Startle Response (110 dB)

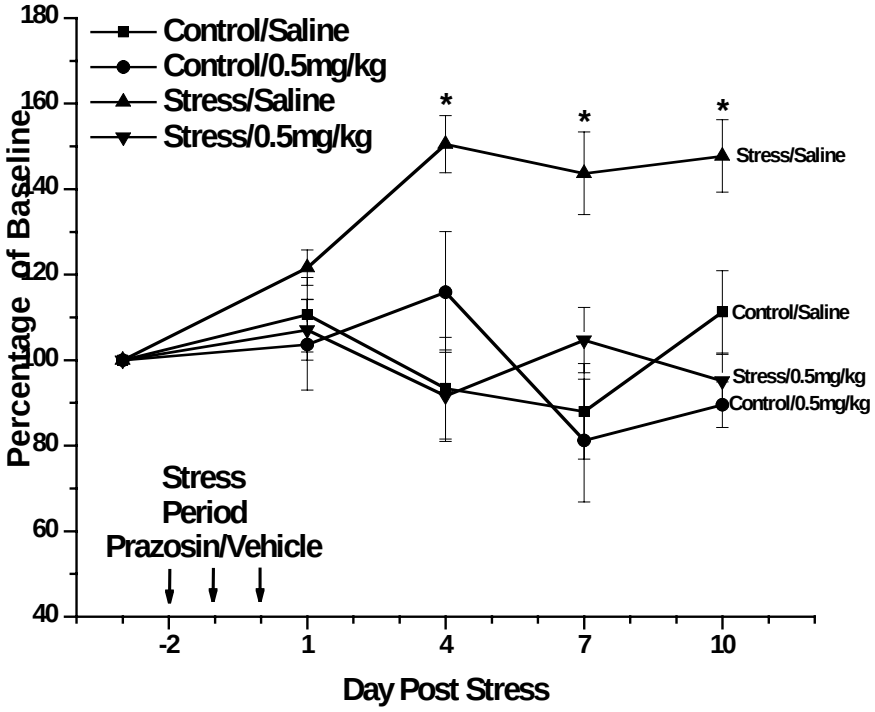

Figure 3 – Percentage of pre-stress baseline of acoustic startle (group means ± SEM) for groups treated with Prazosin (0.5 mg/kg) or vehicle (saline) 30 minutes prior to the stress experience. Asterisks (*) indicate significant differences in mean ASR between stressed, vehicle-treated rats and all other groups (n=12-15 for each group; $p<0.05$).

Prazosin Dosage

Figure 4 summarizes ASR in animals given various doses (0.05, 0.1 and 0.25 mg/kg) of prazosin or vehicle. ANOVAs for repeated measures with factors of stress status and drug dosage and test day as the repeated measure showed an effect of day x stress x drug [$F(16,100)=2.41$, $p=0.016$]. Tukey's post-hoc test on each day indicated that the stress/saline group differed significantly from every other group on day 4 and 10 following stress and from all prazosin treated groups on day 7 following stress ($n=6$ for each group, $p<0.05$).

Acoustic Startle Response (110 dB)

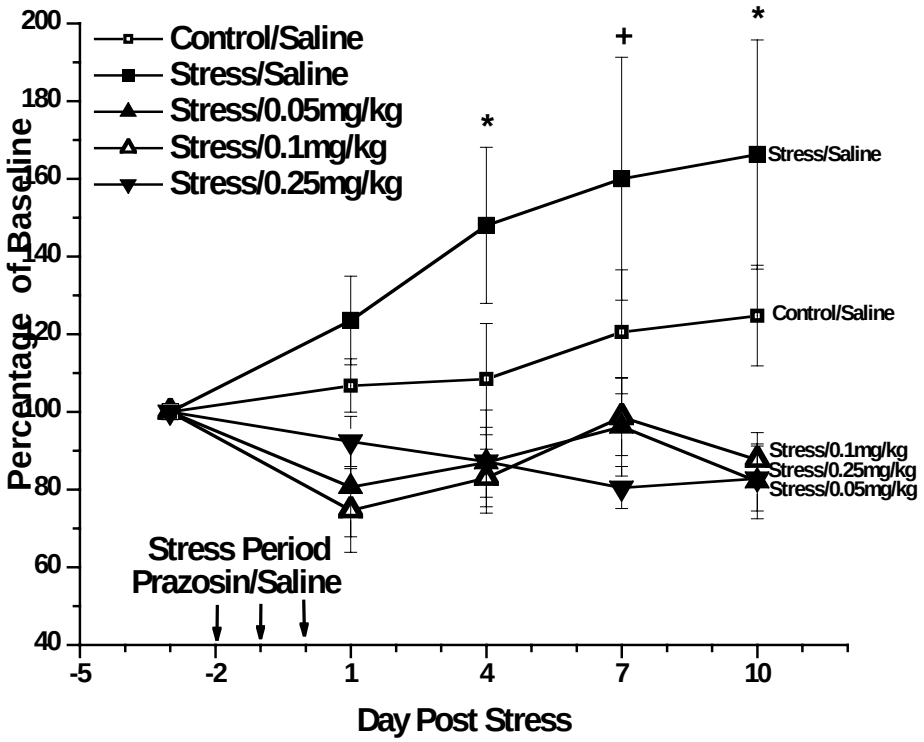

Figure 4 – Percentage of pre-stress baseline of acoustic startle (group means ± SEM) for groups treated with prazosin or saline 30 minutes prior to stress on each of the three day stress procedures. Asterisks (*) indicate significant differences between the stressed animals that received saline compared to all other groups (n=6 for each group; $p<0.05$). Plus sign (+) indicates significant differences between the stressed animals that received saline compared to all prazosin treated groups (n=6 for each group; $p<0.05$).

Discussion

The model of traumatic stress used in this study has previously been shown to result in a number of dysfunctions similar to some symptoms of PTSD in humans, including noradrenergic hyper-responsiveness, memory deficits and exaggerated startle response (Seligman and Maier 1967; Servatius et al. 1995; Ottenweller et al. 1989). While the age of the animals used in this study was younger than those used in previous studies, the same three day protocol of restraint and tailshock shock resulted in similar effects on body weight and stress induced ASR elevation (Figures 1 & 2). Many studies using milder forms of stress (e.g. restraint only) have shown short term effects of stress but fewer long-term, persistent effects; whereas this model of repeated restraint combined with tailshock has been shown to result in persistent physiological and behavioral abnormalities (Braga et al., 2004; Servatius et al., 1995). This paradigm, therefore, is ideal for evaluating pharmacological treatments for traumatic stress that results in persistent pathophysiologies.

Given its ability to cross the blood brain barrier and long history of safe use, prazosin seemed the ideal candidate to use as the antagonist to experimentally test for reduction in α_1 AR mediated stress effects. There is growing evidence to indicate that prazosin may have potential therapeutic value for treatment of PTSD in human populations (Vieweg et al., 2006). It has been shown to improve sleep and reduce nightmares (Taylor & Raskind, 2002; Peskind et al., 2003; Griffith 2005) as well as to reduce distress resulting from trauma specific cues (Taylor et al. 2006). These studies

used dosages of prazosin between 1 and 5 mg/day for adult males. While the pharmacokinetic responses to prazosin in humans and rodents may be different, it is notable that the effective dosages in the human studies were commensurate with the lower levels evaluated in this study (0.05, 0.1 mg/kg). The results of this study give a specific indication that prazosin may also be useful in reducing or preventing pathophysiologies related to traumatic stress when it can be given prior to stressful events.

Pharmacological prevention of stress-related psychiatric disorders such as PTSD is a topic of current medical interest that has received limited attention by clinical and neurobiological investigations (Pitman et al., 2002). Most of the research performed thus far has focused on the effectiveness of a few classes of compounds in alleviating the symptoms of stress-related disorders (for review see Albucher and Liberzon, 2002; Marmar et al., 2002). These include tricyclic antidepressants, monoamine oxidase inhibitors, and serotonin reuptake inhibitors. Although clinical research has shown that these agents can alleviate symptoms and facilitate recovery, their overall efficacy is limited and very often hindered by their serious side effects. The development of more specific pharmacological agents, with potentially less significant side effects, as a therapeutic strategy aimed at preventing the establishment of stress-related disorders is an important step in the treatment of these illnesses. While instances of trauma often can not be foreseen, pretreatment with prazosin may be beneficial in the limited situations when it can be foreseen and pretreatment is logistically feasible, such as disaster response, international aid missions and combat.

In summary, this study found that prazosin can reduce persistently elevated ASR due to traumatic stress, when administered prior to the stressor. This further implicates the role of α_1 adrenoceptors in regulating the response to traumatic stress and its subsequent pathophysiologies. It also offers the potential for preventive, rather than just therapeutic treatment of stress-related anxiety disorders such as PTSD.

Acknowledgements

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CHAPTER 3

Stress Impairs α_{1A} Adrenoceptor Mediated Suppression of LTP in the Basolateral Amygdala

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The basolateral amygdala (BLA) is an area known to be involved in the processing of emotional memory. Synaptic plasticity in the BLA is thought to play a key part in this memory formation and in memory-related pathologies related to stress, such as post-traumatic stress disorder. This study sought to investigate α_{1A} adrenoceptor involvement in long-term potentiation (LTP) in the BLA and to determine the effects of traumatic stress on this type of plasticity. In the rat BLA in vitro, the α_{1A} specific agonist A61603 (1 μM), in the presence of the α_2 adrenoceptor antagonist yohimbine and the β adrenoceptor antagonist propranolol, completely blocked LTP induced by theta-burst stimulation (TBS) in controls. In the BLA of animals previously exposed to a repeated restraint and tailshock stress protocol it was still possible to induce LTP in the presence of A61603, though at a significantly reduced level of potentiation than in the absence of any drug. The suppression of LTP in the BLA of control animals was eliminated in the presence of the α_{1A} specific antagonist WB4101 (1 μM), while the antagonist had no effect on the induction of LTP, nor the level of potentiation in the BLA of stressed animals. Given the likely role of LTP in memory formation, these findings suggest involvement of α_{1A} adrenoceptors in certain stress related affective disorders and potentially a specific pharmacological target in the α_{1A} adrenoceptors for treatment of these disorders.

Keywords: Stress, basolateral amygdala, LTP, α_{1A} adrenoceptors, norepinephrine.

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Introduction

Extreme or chronic stress can cause a variety of detrimental effects that may lead to lasting pathophysiologies and emotional memory (Vermetten and Bremner, 2002; McEwen, 2002). The basolateral amygdala (BLA) is a key component of the brain's neuronal network that determines the emotional significance of external events and is involved in the formation, processing and storage of emotional memory (Davis et al., 1994; LeDoux, 2000; Buchel et al., 1998; Davidson 2002; Buchel and Dolan, 2000). Via efferent pathways to the hypothalamus (Goldstein et al., 1995; Pitkänen et al. 2000), the BLA can trigger the neuroendocrine cascades that are part of the stress response (Habib et al., 2001; Davis, 1992; Cahill and McGaugh, 1996).

One of the neurotransmitters known to be involved in the modulation of plasticity associated with memory is norepinephrine (NE). During stress, there is a strong enhancement of NE release in the BLA (Galvez et al., 1996; Stanford, 1995; Quirarte et al., 1998; Tanaka et al., 2000). The α_1 ARs have been shown to have some involvement in the amygdala's response to stress and processing of emotional memory (Ferry et al., 1999). In the central and basolateral nuclei, α_{1A} ARs are expressed in moderate to high levels, whereas α_{1B} and α_{1D} receptors are expressed at low levels (Day et al., 1997). It has previously been shown that repeated restraint and tailshock produce a severe impairment in the α_{1A} AR-mediated facilitation of GABA release in the BLA, indicating that stress impairs the function of α_{1A} ARs (Braga et al., 2004). It has additionally been established

that ARs can become desensitized after prolonged exposure to agonists (Yang et al., 1999; Chalothorn et al., 2002).

Synaptic plasticity is thought to underlie memory formation and takes several forms including long-term potentiation (LTP) and long-term depression (LTD). Since the discovery of LTP, the hippocampus has been the primary focus of research into memory and synaptic plasticity. This has enabled researchers to uncover a number of different aspects of the mechanisms underlying LTP in the hippocampus (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). It has also become apparent that stress can affect plasticity in hippocampus. Studies have shown that certain types of stress can reduce or eliminate LTP in that region (Kim et al., 1996; Diamond et al., 2004). While there are some similarities in the general processes of synaptic plasticity in the hippocampus and the amygdala, recent findings show significant differences between the mechanisms underlying plasticity in these two areas, such as roles of certain receptors and signaling mechanisms (Chapman et al., 2003). Differences have also been seen in the effects of stress on plasticity in these two regions (Vouimba et al., 2004; Diamond et al., 2004; Akirav et al., 2001). This study sought to investigate α_{1A} adrenoceptor involvement in the modulation of LTP in the BLA and to determine the effects of traumatic stress on this type of plasticity. It was observed that α_{1A} AR activation could mediate the suppression of the induction of LTP. Exposure to traumatic stress appeared to partially block this suppression.

Materials & methods

Experimental design and stress protocol

All animal experiments were performed in accordance with our institutional guidelines after obtaining the approval of the Institutional Animal Care and Use Committee. 37 animals were used in 7 different experimental treatments: 22 control (5 no α_{1A} drug, 5 A61603, 5 A61603 and WB4101 and 7 A61603 with upward baseline shift) and 15 stressed (5 no α_{1A} drug, 6 A61603 and 4 A61603 and WB4101). Male, 17 day old Sprague Dawley rats were received and housed in a climate-controlled environment on a 12 hr light/dark cycle (lights on at 7:00 A.M). At 21 days old following weaning they were randomly divided into control and stressed groups. They were housed individually, with food and water supplied ad libitum. The stressed group was exposed to stress for three consecutive days beginning at 22 days old. Rats were sacrificed and brain slices were prepared within 24 hours following the final stress session. Age and sex were chosen to match our previous investigations of the effects of the α_{1A} specific agonist A61603 on neuronal activity in the BLA (Braga et al., 2004). The experiments were performed in a blind fashion until the data were analyzed.

Stress exposure consisted of a two-hour per day session of immobilization and tail-shocks on three consecutive days. The animals were stressed in the morning (between 8 AM and 12 PM). They were restrained in a cloth wrap with their head and torso immobilized in a Plexiglas tube while 40 electric shocks (2 mA, 3 s duration) were

applied at varying intervals (140 to 180 s) using an electrode attached to the tail. This stress protocol was adapted from the learned helplessness paradigm in which animals undergo an aversive experience under conditions in which they cannot perform any adaptive response (Seligman and Maier 1967; Seligman and Beagley 1975). We stressed the rats for three consecutive days because it had been previously demonstrated that repeated stress sessions for three days is more effective than a single stress session in producing physiological and behavioral abnormalities, such as elevations in basal plasma corticosterone levels, exaggerated acoustic startle responses and reduced body weight (Servatius et al. 1995; Ottenweller et al. 1989). More stress sessions, beyond the three days, do not produce greater physiological and behavioral changes (Servatius et al. 1995; Ottenweller et al. 1989). Control animals remained in their home cages.

Slice preparation

The amygdala slice preparation has been described previously (Li et al. 2001). Briefly, the rats were anesthetized with halothane and then decapitated. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 11 glucose, bubbled with 95% O_2/5%CO_2. A block containing the amygdala region was prepared by rostral and caudal coronal cuts, and coronal slices, 400 μm thick, were cut using a Vibratome (series 1000, Technical Products International, St. Louis, Missouri). Slices were kept in a chamber containing oxygenated ACSF at room temperature, and experiments started ≥1 hour after slice preparation.

Electrophysiology

For field potential recordings, slices were transferred to an interface-type recording chamber maintained at 32°C, where they were perfused with ACSF at 0.7-1 ml/min. Field potential was amplified with a differential amplifier (Warner Instrument Corp.). The output was digitized with a Digidata 1200 interface (Axon Instruments). On- and off-line data acquisition and analysis was carried out using WCP version 1.7b (John Dempster, University of Strathclyde, Glasgow, UK). Baseline intracellular and field recordings were established for 20 min before application of theta burst stimulation. EPSP slopes and amplitudes were normalized to this averaged baseline value (100%). The maximum rate of EPSP within 10% - 90% rising phase was measured as the slope of EPSP using the WCP software.

Stimulation

Synaptic responses were evoked with sharpened tungsten bipolar stimulating electrodes (World Precision Instruments, Sarasota, Florida) placed in the external capsule (EC). The stimulating electrode was ~2 mm from the recording site. Stimuli were delivered using a photoelectric stimulus isolation unit having a constant current output (ISO-Flex; Stimulus Isolation Unit, Jerusalem, Israel). The stimulus intensity was adjusted to produce a synaptic response of less than 50% of the maximum amplitude

obtainable without triggering an action potential response. Peak response amplitudes were measured with respect to the resting membrane potential. Single 0.1-msec monophasic square pulses were applied continuously throughout the experiment at 0.1 Hz. To induce synaptic plasticity, theta burst stimulation (TBS): a brief, high frequency pulse train of 5 pulses at 100 Hz given at the theta-rhythm, 5Hz, for 4 sec) was applied at the same intensity through the same electrode as used for the test stimulation. The theta burst stimulation protocol mimics the typical firing mode of pyramidal cells during learning (Otto et al., 1991). The second TBS after ten minutes has been shown to induce NMDA-dependent LTP in the BLA following a short term potentiation by the initial TBS (Chen et al., 2003, Li et al., 1998). Field potentials were filtered at 1 kHz, and digitized on line at 5 kHz.

Analysis

All data are presented as mean ± SEM. Sample size n refers to the number of slices. From each rat, a maximum of two slices were used for each set of experimental variables. Slices from individual rats were averaged. Student's t-tests were used to compare groups (independent t-tests) as well as individual treatment groups to their baseline (paired t-tests). Means of final ten time points and initial ten time points (baseline) were used for these comparisons.

Drugs

The following drugs were used: DL-propranolol (Sigma-Aldrich, St. Louis, MO), a β adrenoceptor antagonist; yohimbine hydrochloride (Sigma-Aldrich, St. Louis, MO), an α_2 adrenoceptor antagonist; N-[5-(4,5-Dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methanesulfonamide hydrobromide (A61603, Tocris Cookson, Ballwin, Missouri), a selective α_{1A} agonist (Knepper et al., 1995); 2-(2,6-Dimethoxyphenoxyethy) aminomethyl-1,4-benzodioxane hydrochloride (WB4101, Tocris Cookson, Ballwin, Missouri), a selective antagonist of the α_{1A} adrenoceptor (Zhong and Minneman 1999).

Results

In control animals (Figure 1), dual theta-burst stimulation (TBS) induced an LTP in the BLA field potential. The dual TBS-induced synaptic potentiation remained at significantly higher levels for more than 30 min [$t(4)=7.09$, $p=0.002$] in the presence of β adrenoceptor antagonist propranolol (10 μM) and α_2 adrenoceptor antagonist yohimbine (20 μM). The slope of field-recorded excitatory post-synaptic potentials (fEPSPs) 30 min after the second TBS was 141.2 ± 11.1% (n=7) of the initial baseline values. In order to determine the effects of α_{1A} AR activation on induction of LTP, a bath application containing α_{1A} specific agonist A61603 (1 μM) along with β adrenoceptor antagonist propranolol (10 μM) and α_2 adrenoceptor antagonist yohimbine (20 μM) was done for 10-20 min until recording levels stabilized. In the BLA of control animals, the dual TBS-induced synaptic potentiation returned to baseline levels within 30 min [$t(4)=0.87$,

p=0.435], indicating that α_{1A} AR activation blocks LTP in the BLA of control animals. The slope of fEPSPs 30 min after the second TBS was 109.2 ± 10.0% (n=6) of the initial baseline values. An independent t-test showed a significant difference between the two groups [t(8)=3.95, p=0.04].

Control/No Drug and Control/A61603

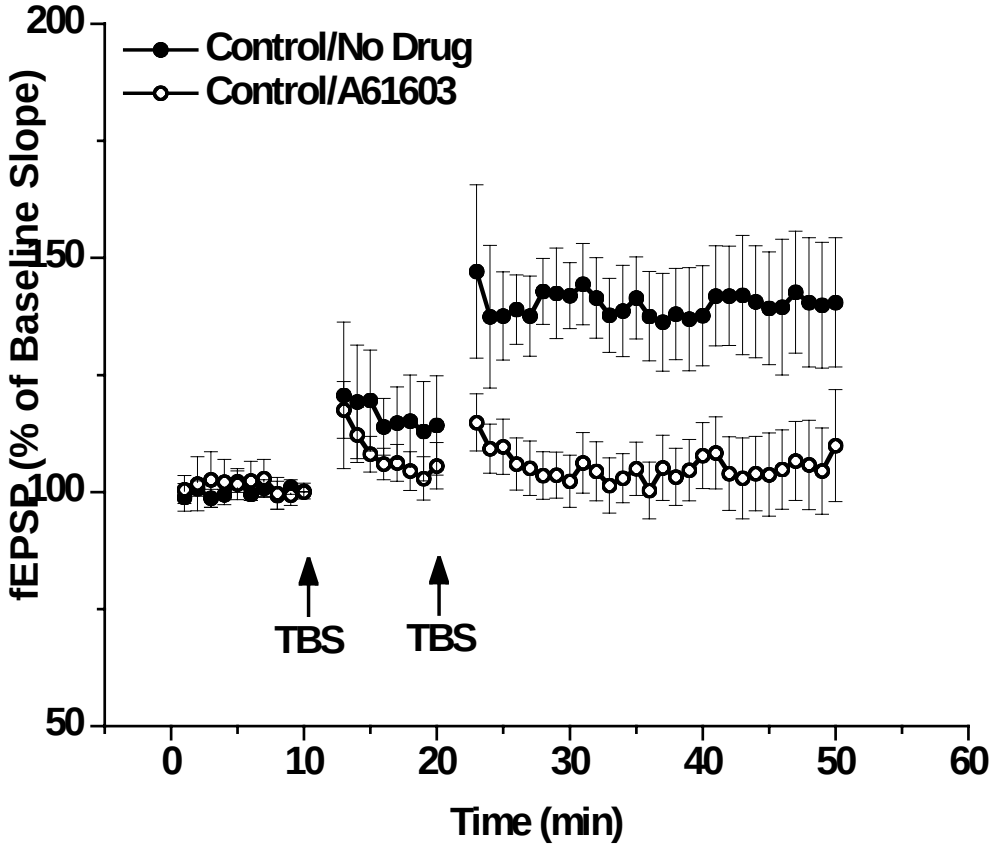

Figure 1. Experiments were done in the presence of propranolol (10 μM) and yohimbine (20 μM). LTP is induced by dual TBS of external capsule in control (n=7). Activation of α_{1A} adrenoceptors (A61603, 1 μM) blocks induction of LTP by two TBS in control animals (n=6). The values are expressed as a percentage of the mean of six responses at 0.1 Hz before the application of TBS; $p<0.05$. Each point represents the mean ± SEM. TBS was applied at times shown by arrows.

In the BLA of stressed animals with no α_{1A} drug treatment (Figure 2) the dual TBS-induced synaptic potentiation remained at significantly higher levels for more than 30 min [$t(4)=6.87$, $p=0.002$]. The slope of fEPSPs 30 min after the second TBS was 155.4 ± 15.2% (n=7) of the initial baseline values. In a bath application containing the α_{1A} specific agonist A61603 (1 μM) along with β adrenoceptor antagonist propranolol (10 μM) and α_2 adrenoceptor antagonist yohimbine (20 μM), following stabilization, the dual TBS-induced synaptic potentiation remained at significantly higher levels for more than 30 min [$t(5)=3.15$, $p=0.025$]. The slope of fEPSPs 30 min after the second TBS was 122.1 ± 11.8% (n=6) of the initial baseline values. However, α_{1A} AR activation with A61603 reduced, but did not completely block, the induction of LTP.

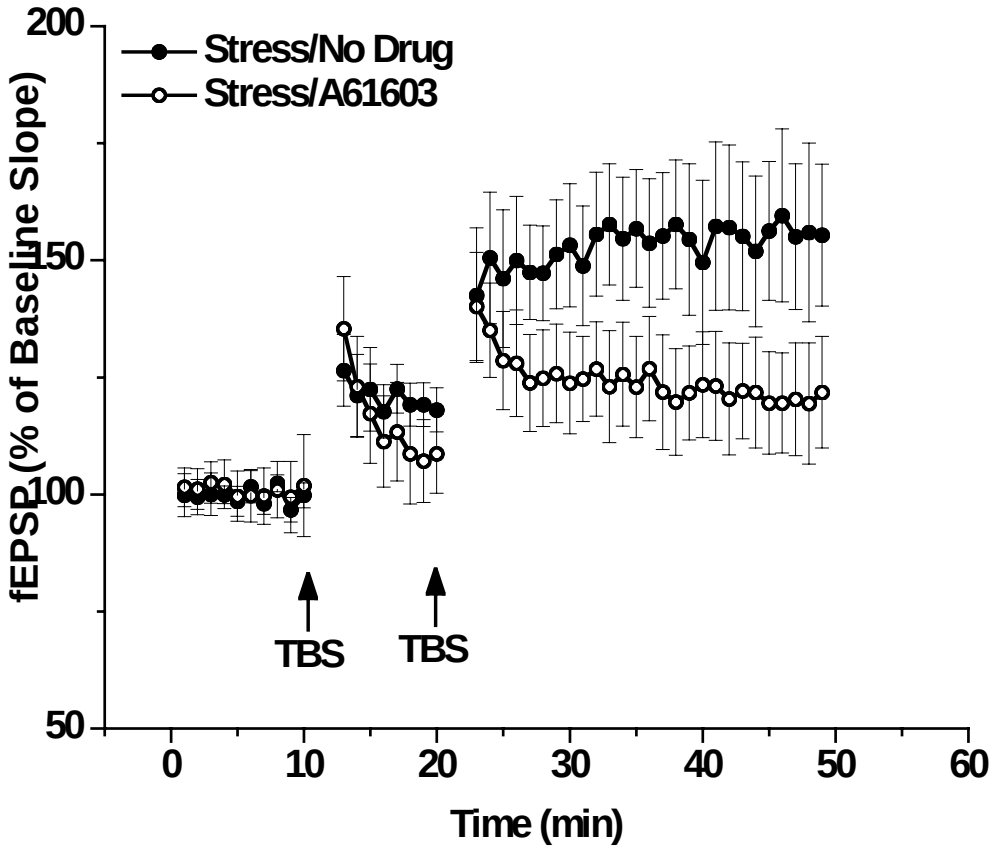

Figure 2. Experiments were done in the presence of propranolol (10 μM) and yohimbine (20 μM). LTP is induced by dual TBS of external capsule in stressed animals (n=7). Stress reduces, but does not eliminate, LTP in the presence of α_{1A} adrenoceptor activation (A61603, 1 μM) (n=6). The values are expressed as a percentage of the mean of six responses at 0.1 Hz before the application of TBS. Each point represents the mean ± SEM; $p<0.05$. TBS was applied at times shown by arrows.

To examine whether blocking α_{1A} AR activation would allow for dual TBS induction of LTP in control animals, an α_{1A} specific antagonist WB4101 (1 μM) was added to the bath prior to A61603 (1 μM) along with β adrenoceptor antagonist propranolol (10 μM) and α_2 adrenoceptor antagonist yohimbine (20 μM) (Figure 3). Subsequently, in control animals, dual TBS was able to induce potentiation significantly higher than baseline for more than 30 min. The slope of fEPSPs 30 min after the second TBS was 136.9 ± 14.4% (n=9) of the initial baseline values. Compared to controls treated with agonist, A61603, but not the antagonist, WB4101, an independent t-test revealed a significant effect [$t(8)=2.41$, $p=0.042$].

Control/A61603 and Control/A61603&WB4101

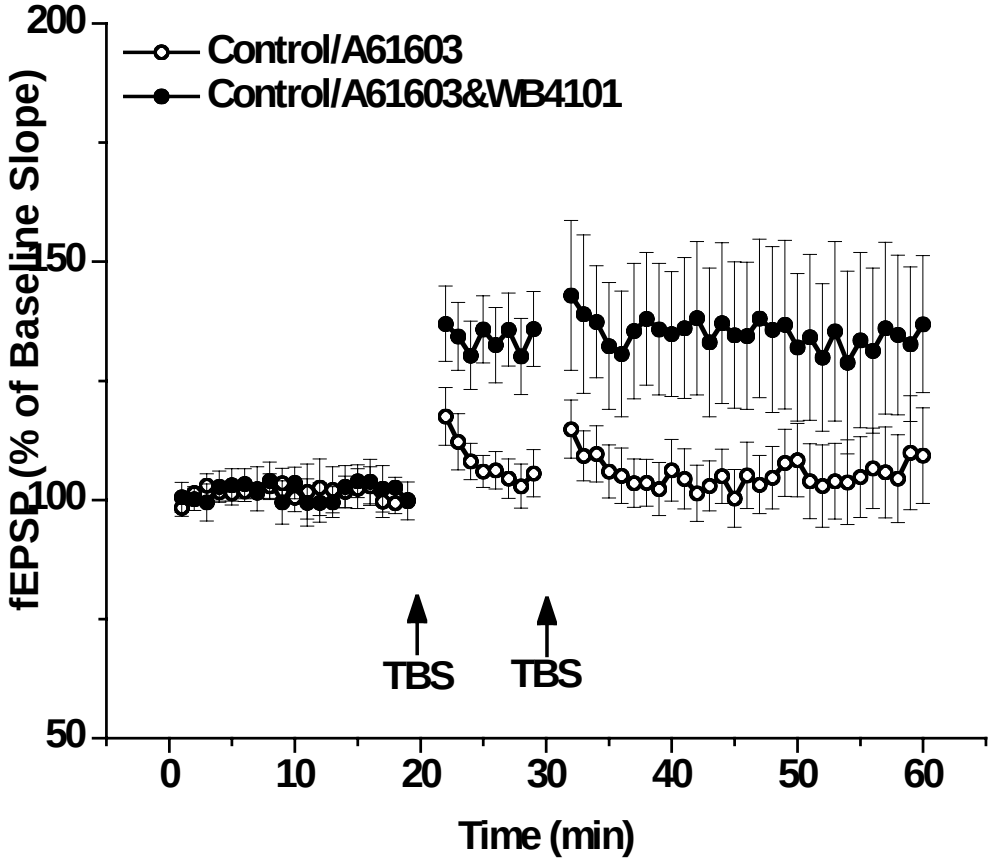

Figure 3. Experiments were done in the presence of propranolol (10 μM) and yohimbine (20 μM). The presence of α_{1A} adrenoceptor antagonist WB4101 (1 μM) eliminates α_{1A} adrenoceptor-mediated (A61603, 1 μM) blockade of the induction of LTP by dual TBS in control animals (n=9). The values are expressed as a percentage of the mean of six responses at 0.1 Hz before the application of TBS. Each point represents the mean ± SEM; $p<0.05$. TBS was applied at times shown by arrows. Control/A61603 data is identical to that compared in Figure 1.

In stressed animals (Figure 4), the presence of both agonist and antagonist had no effect compared to agonist alone on the ability of dual TBS to induce LTP. The slope of fEPSPs 30 min after the second TBS was 127.6 ± 13.2% (n=6) of the initial baseline values. Compared with slices treated with agonist alone, no significant difference was seen [$t(8)=0.33$, $p=0.75$].

Stress/A61603 and Stress A61603&WB4101

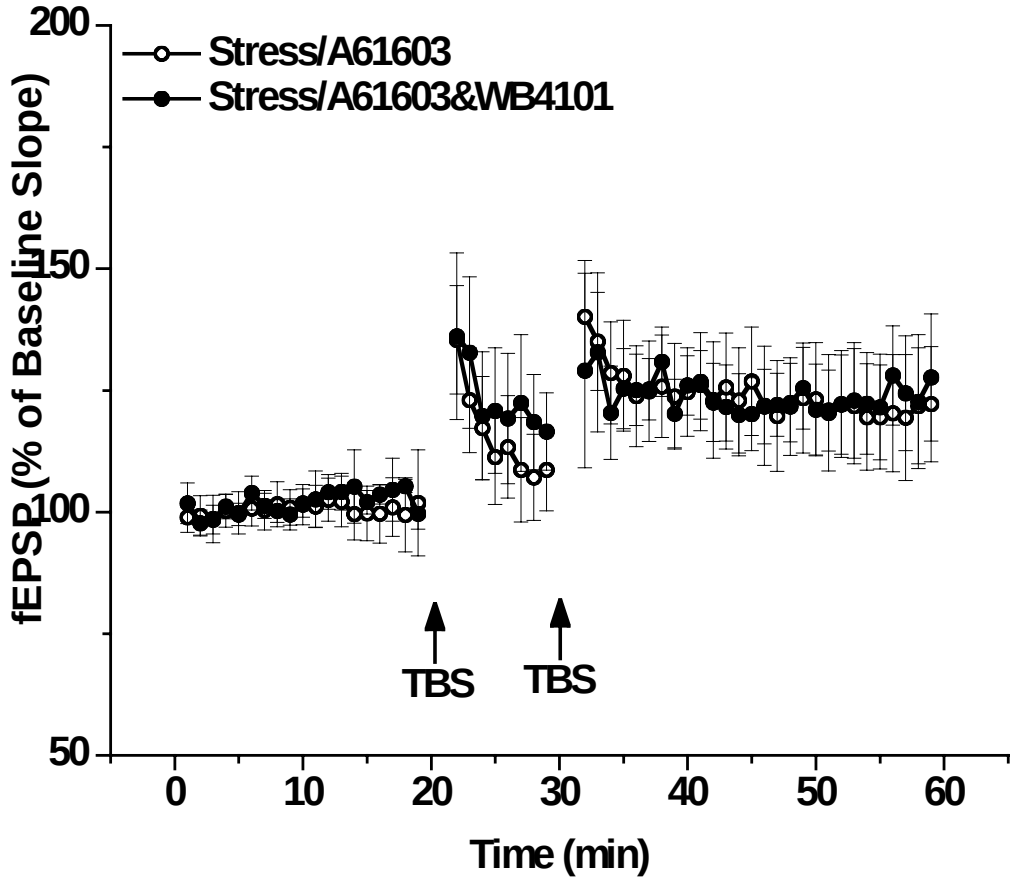

Figure 4. Experiments were done in the presence of propranolol (10 μM) and yohimbine (20 μM). Induction of LTP by two TBS in stressed animals (n=6) in the presence of α_{1A} adrenoceptor agonist (A61603, 1 μM) is unaffected by the addition of α_{1A} adrenoceptor antagonist WB4101 (1 μM). The values are expressed as a percentage of the mean of six responses at 0.1 Hz before the application of TBS. Each point represents the mean ± SEM. TBS was applied at times shown by arrows. Stress/A61603 data is identical to that compared in Figure 2.

Since it was known that application of the α_{1A} specific agonist A61603 (1 μM) facilitates GABAergic transmission in the BLA, resulting in an overall suppression of fEPSPs in the BLA of control animals but not stressed animals (Braga et al., 2004), it was necessary to determine whether this suppression alone was responsible for completely blocking induction of LTP in controls. To investigate this, following the stabilization of potentials at the suppressed level from the introduction of A61603, the peak was adjusted to match previous levels (Figure 5). Dual TBS was then again used to attempt to induce LTP in the presence of A61603 (1 μM) along with the β adrenoceptor antagonist propranolol (10 μM) and the α_2 adrenoceptor antagonist yohimbine (20 μM) in the BLA of control animals. With the upward shift of the baseline, dual TBS failed to induce significantly higher potentiation for more than 30 min [$t(6)=0.57$, $p=0.591$]. The slope of fEPSPs 30 min after the second TBS was 109.3 ± 10.0% (n=6) compared to the average upwardly shifted baseline levels of 106.3 ± 13.5% of the initial baseline values [$t(10)=0.21$, $p=0.833$].

Control with Upward Shift/A61603 and Control/A61603

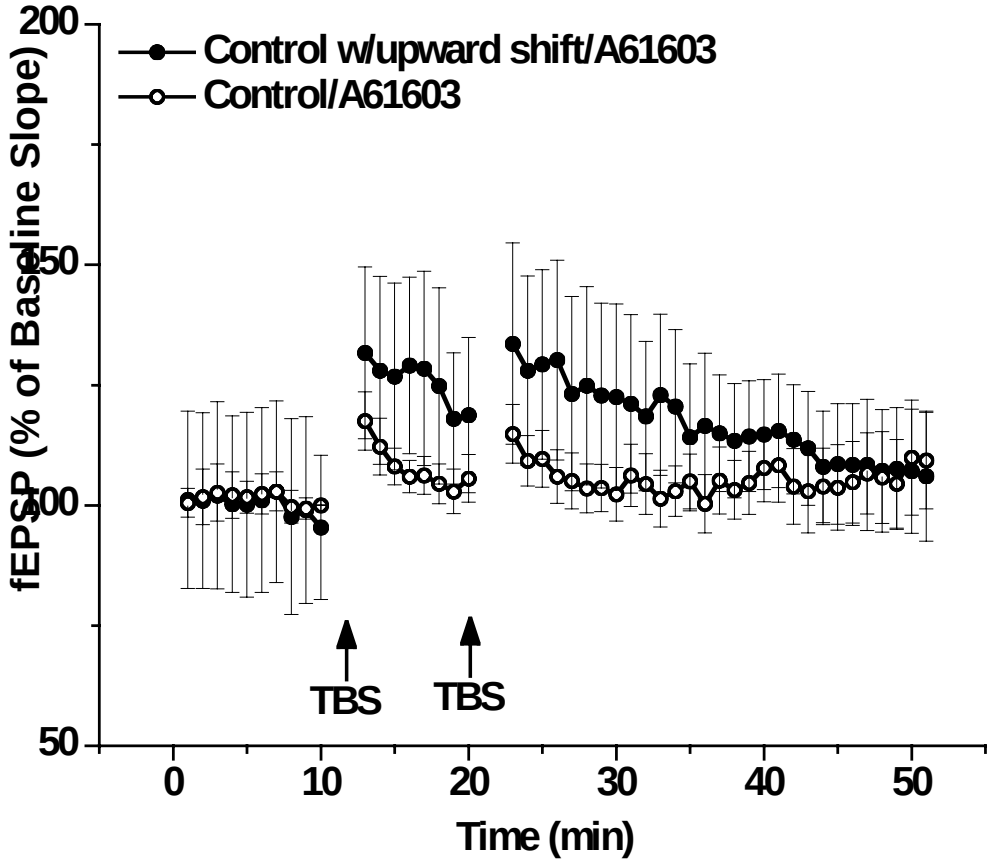

Figure 5. Experiments were done in the presence of propranolol (10 μM) and yohimbine (20 μM). Activation of α_{1A} adrenoceptors (A61603, 1 μM) blocks induction of LTP by dual TBS in control animals (n=6), even after suppression is offset by increased stimulation. The values are expressed as a percentage of the mean of six responses at 0.1 Hz before the application of TBS. Each point represents the mean ± SEM. TBS was applied at times shown by arrows. Control/A61603 data is identical to that compared in Figure 1.

Discussion

In this study, it was seen that while LTP could be induced in both control and stressed animals, activation of α_{1A} ARs resulted in the blocking of induction of LTP in control animals. In animals that had undergone restraint and tail shock stress, LTP in the BLA could still be induced despite the activation of α_{1A} ARs. These findings indicate a role for α_{1A} ARs in the amygdala in the stress response, as well as suggest a reduced sensitivity to α_{1A} in the amygdala following exposure to traumatic stress. They also provide one possible explanation for why individuals who have previously been exposed to stress are more likely to form strong or even pathological emotional memories of subsequent stressful events.

Afferents to the amygdala from the LC cause a significant increase in NE infusion into the amygdala following stress. It is known that this increased NE plays a role in affecting emotional memory (Stanford, 1995). The α_1 ARs have been shown to also mediate NE's effect on emotional memory, through interaction with β ARs (Ferry et al., 1999). Here we have found evidence of an α_{1A} specific effect in the BLA following stress.

Previous studies using the repeated restraint and tail shock model of traumatic stress used here have shown it to result in increased plasma corticosterone levels (Servatius et al., 1995). It is notable that glucocorticoid receptors have been shown to be coexpressed with α_1 ARs (Fuxe et al., 1985) and that corticosterone down regulates α_1

ARs (Stone et al., 1987; Joels and de Kloet, 1989). Increased corticosterone following stress is therefore another possible mechanism for the altered sensitivity and function of α_{1A} ARs in the BLA following stress.

Though conclusive causal evidence of an LTP-memory link remains elusive, numerous previous studies have presented evidence closely tying the two and have implicated LTP as the mechanism by which emotional learning and memory is mediated in the amygdala. Determining how this plasticity is modulated by stress might deliver insight into what mechanisms underlie the pathological long term effects that traumatic or repeated stress can sometimes cause. Identifying these mechanisms would allow for more focused and effective treatments for stress-related affective disorders such as PTSD.

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CHAPTER 4

Discussion

The primary finding shown in Chapter 2 was that while stress increased ASR compared to controls, prazosin treatment prior to stress reduces this elevated ASR induced by stress. This reduction was seen at doses ranging from 0.05 to 0.5 mg/kg. Prazosin's ability to cross the blood brain barrier and long history of safe use, make it the ideal candidate to use as the antagonist to experimentally test for reduction in α_1 AR mediated stress effects.

Clinical and neurobiological investigations into the pharmacological prevention of PTSD have only recently begun to receive attention by the medical community (Pitman et al., 2002). Most of the previous research has focused on a few classes of compounds, tricyclic antidepressants, monoamine oxidase inhibitors, and serotonin reuptake inhibitors, in alleviating the symptoms of stress-related disorders (Albucher and Liberzon, 2002; Marmar et al., 2002). These agents can alleviate symptoms and facilitate recovery, but their effectiveness is limited given the seriousness of associated side effects. Developing more specific pharmacological agents, with fewer and less significant side effects is an important step in the treatment of PTSD. Recent studies indicate that prazosin has potential therapeutic value for treatment of PTSD in human populations by improving sleep and reducing nightmares (Taylor & Raskind, 2002; Peskind et al., 2003; Griffith 2005) as well as to reducing distress resulting from trauma specific cues (Taylor et al. 2006).

In Chapter 3, we revealed a role for α_{1A} ARs in alterations in neuroplasticity in the BLA following stress. A61603, an α_{1A} AR agonist, completely blocked induction of LTP in the BLA of control animals, while only partially impairing LTP in the BLA of stressed animals. In controls, this blocking effect was eliminated in the presence of WB4101, an α_{1A} AR antagonist, whereas no effect of the presence of the antagonist was seen on the partial impairment of LTP by the agonist in the BLA of stressed animals.

There is a significant increase in NE infusion into the amygdala following stress from the LC. This increased NE has a role in affecting emotional memory (Stanford, 1995), and the α_1 ARs have been shown to also mediate NE's effect on emotional memory, through interaction with β ARs (Ferry et al., 1999). Our investigations suggest that NE, acting via α_{1A} adrenoceptors, blocks the induction of LTP in the BLA. Based on previous studies (Braga et al., 2004) it is possible that the α_{1A} adrenoceptor-mediated blockade of LTP is due to the facilitation of GABAergic synaptic transmission induced by NE. By using the inescapable tail-shock model of traumatic stress in rats, our investigations showed that stress impaired the α_{1A} adrenoceptor-mediated modulation of LTP. This impairment could result from receptor desensitization, internalization, or downregulation. Adrenergic receptors desensitize or undergo downregulation following prolonged exposure to the agonist (Yang et al., 1999). During stress exposure, excessive release of NE in the amygdala (Galvez et al., 1996; Quirarte et al., 1998; Tanaka et al., 2000) may be responsible for the impairment of α_{1A} AR function. Administration of α_{1A} adrenoceptor antagonists prior to a period of intense stress, to block/protect these

receptors from excessive NE activation, will reduce stress-induced alterations in amygdala's neuroplasticity.

It is also interesting to note that the level of LTP was reduced, but not eliminated, in the BLA of the stressed rat in the presence of the α_{1A} adrenoceptor agonist; unlike in the control BLA, the additional presence of the antagonist does not return the LTP level to normal. While further study is needed to better understand this, it may be due to a loss of affinity of the α_{1A} adrenoceptors for the antagonist. Intra-basolateral amygdala infusions of phenylephrine (a non-selective α adrenoceptor agonist) at low doses has been shown to impair memory retention (Ferry et al., 1999). A61603 has been shown to be at least 35-fold more potent at α_{1A} adrenoceptors than at α_{1B} or α_{1D} sites as examined in radioligand binding assays and in isolated canine prostate strip preparations (Buckner et al., 1996; Knepper et al., 1995). The unique features of A61603 may prove to be a useful probe for studies of α_{1A} adrenoceptor regulation of brain functions.

Fear-conditioning or exposure to a traumatic event produces long-lasting changes in the efficacy of synaptic transmission in the amygdala (Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Adamec et al., 2001; LeDoux, 1992; Davis, 1994; Maren et al., 2001). Thus, during exposure to traumatic events, excessive release of NE in the amygdala (Galvez et al., 1996; Quirarte et al., 1998;Tanaka et al., 2000) may be responsible for facilitating or damping emotional learning and memory due to differential activation of β or α ARs. In the basolateral amygdala, NMDA receptor dependent LTP, a leading candidate for a memory mechanism, can be induced by administration of

multiple high frequency stimulations delivered at intervals of a few seconds to a few minutes following the initial tetanic stimulation (Aroniadou-Anderjaska et al., 2001; Huang and Kandel, 1998; Maren, 1996). The TBS protocol was used in the current study to mimic the typical firing mode of pyramidal cells during learning (Otto et al., 1991). The second theta burst stimulation was selected to be delivered at 10 minutes after the initial stimulation because the amplitude of field potential generally returned more closely to the baseline levels during that time interval and because neuronal firing frequency is not always reproducible at higher frequency during short multi-second intervals (except in pathological conditions such as epilepsy). It has been shown that a single high frequency tetanus or TBS stimulation induces a short-term potentiation in BLA-external capsule synaptic transmission lasting approximately 10 minutes (Chen et al., 2003; Li et al., 1998).

Under physiological conditions, basal levels of NE, acting predominately via α_{1A} adrenoceptors, contribute to tonic inhibition of pyramidal neurons in the BLA to prevent over excitation. When the amygdala is activated in response to an emotionally significant event, triggering the release of larger amounts of NE from the LC, activation of both β and α adrenoceptors will facilitate both excitatory and inhibitory transmission in the neuronal circuits. Thus, the neuronal activities are able to operate at higher levels with a balanced excitatory and inhibitory influence. Therefore, the α_{1A} adrenoceptor-mediated suppression of synaptic plasticity may prevent emotional memory processing under stressful conditions. It should be considered, however, that the net effect of stress on the function of the noradrenergic system in the BLA remains to be determined, as stress may

also induce changes in the interaction of NE with other adrenoceptor subtypes (β and α_2) or other neurotransmitter systems.

Numerous previous studies have presented evidence closely tying LTP and memory and have implicated LTP as the mechanism by which emotional learning and memory is mediated in the amygdala (Maren, 1999). Determining how this plasticity may be modulated by stress could deliver insight into what mechanisms underlie the pathological long term effects that traumatic or repeated stress can sometimes cause. Identifying these mechanisms might allow for more focused and effective treatments for stress related affective disorders such as PTSD.

Implications and Future Directions. The key findings of this project, that α_{1A} ARs play a role in the stress effects on neuronal plasticity of the amygdala and that α_1 AR antagonist prazosin given before stress is able to reduce some heightened behavioral responses to stress, give the prospect of a better understanding of the mechanisms underlying the stress response and a potential preventative measure to some pathological conditions that result from traumatic stress. While more follow up study is warranted, to confirm these findings and better elucidate the α_1 AR's role in the emotional memory processing in the amygdala, it is tempting to consider potential clinical applications of certain α_1 AR specific drugs.

The clinical prospects for the finding that prazosin may block certain pathological aspects that develop from exposure to traumatic stress is even more immediate, given

prazosin's long, safe history of use for hypertension and its relatively limited side effects. It would not require very much additional testing before being approved for this new use. Furthermore, it is already successfully being used to reduce the effects of traumatic stress in PTSD patients, eliminating nightmares, sleep problems and reducing avoidance of stress related cues at a dosage comparable or lower to those seen to be effective in rats in this study (~0.02-0.15 and 0.05-0.5 mg/kg, respectively) (Raskind et al., 2002; Peskind et al., 2003; Taylor et al., 2006).

While both prazosin and the β AR antagonist propranolol are being studied for use in reducing the symptoms of PTSD (Taylor et al., 2006), or even in use for preventative intervention shortly after stress for propranolol (McGaugh, 2004), it would be novel to attempt to use prazosin, prior to the exposure to the stressor, to reduce or prevent some of the effects of exposure to traumatic stress. Even extrapolating these results to humans and exploring the potential for reduction of a more widespread group of stress induced alterations (a considerable though not inconceivable connection), there would still be some problems with this type of usage of prazosin, both due to the sometimes unpredictable nature of traumatic stress, as well as with potential side effects associated with prazosin.

Traumatically stressful events are more often than not, unpredictable. No one expects many of the events that can result in PTSD. But there are some events, known to cause PTSD at high levels, where it would also be logistically feasible to titrate appropriate levels of prazosin in advance. Members of the Armed Forces in certain

combat situations, first responders to disaster sites and international aid workers going into war torn areas are all examples of people going into foreseeable high PTSD-risk situations.

A second problem to arise from the potential pre-stress use of prazosin in humans is the danger of side effects. While relatively low, especially with the low dosages known to treat some symptoms of PTSD, the associated hypotension and hypotonicity may still be a problem for some situations. For example, those in combat situations must often operate at peak cardiovascular performance. This problem has the potential to be offset with complementary, peripherally acting stimulants. Additionally, more specific agents with fewer side effects could also minimize problems with side effects.

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